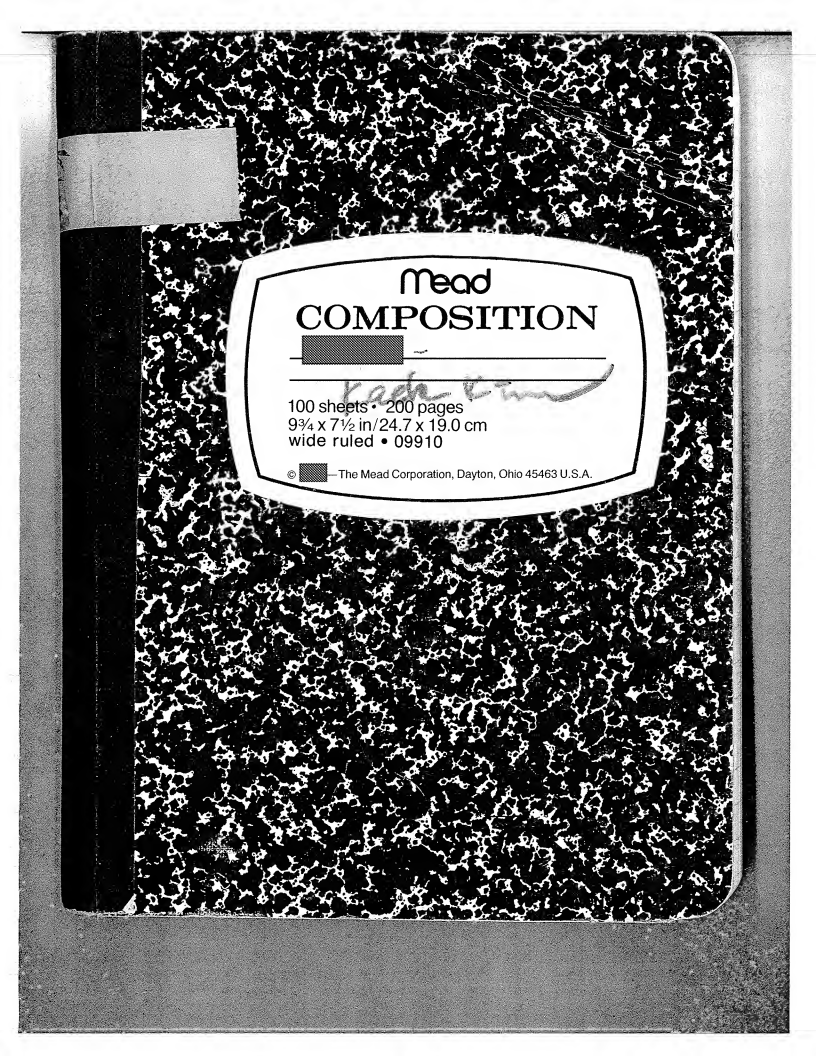
EXHIBIT 18


Mead
COMPOSITION
100 sheets • 200 pages
9¾ x 7½ in/24.7 x 19.0 cm
wide ruled • 09910
© —The Mead Corporation, Dayton, Ohio 45463 U.S.A.

pmel 17 → ← 1st PCR
→ ← 2nd PCR

PCR c̄ human melanocyte RNA — chicken gene homologous to pmel 17 : Japan

700, (600, 400, 300 bp

? — become smaller (600 bp) when cloned

pMEL17

pll247 c̄ deletion — [same donor site / differ acceptor "]

run gel [700]

(☆) get 700 !! → 900 bp

look p 26

4-1BB 1 → ← 2 ① 3F → ← 3R ②

(☆) get Jurkat 500 → (filter already made — Southern [human, Gibbon, mouse DNA / Genomic DNA cut c̄ RI] high stringent

(500) | cloned partially seq.

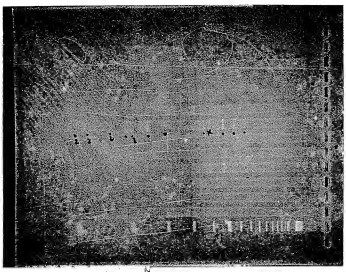
380 | 380 → cloned but (?) PHA-stimulated human PBL T cell

300 | 300 Ribosomal binding protein

| 200 → (?)

Jurkat | Gibbon

① MLA poly A+ (Gibbon T cell)
② Jurkat (human T)
③ Molt4 (human T)



58
Neg Control
MLA poly tr H2
" " H3R
" " 2+3B
" Total H2
" " H3R
" " 2+3B
Move 4 Total H2
" " H3R
" " 2+3B
1000 bp ladder

KWON000131

4-18-89

MLA poly A+ 1 + 2
" " 1 + 3R
" " 2 + 3Th

" Total RNA 1 + 2
" " 1 + 3R
" " 2 + 3Th

Molt 4 "
" "
" "

R8 ~~poly A+~~ Total RNA 1 + 2

Negative control

10 µl each, 150 ~ 400 bp

15 × 20 cm gel (Bio-Rad) in TBE: for 150 3 hr 45 min
for 400

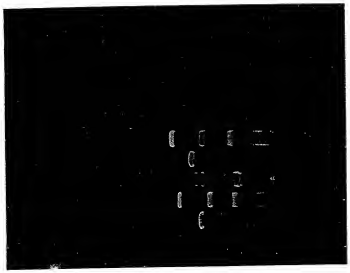
1% Agarose
1.5% Sea Plaque

run until front dye is out

start 12:20 at 104 V 50 mA
12:45 106 V 56 mA
5
18:00 staining (for 30 min)
18:30 destaining

19:30

KWON000132



Vector preparation

pXM ~~(by CDM7)~~ cut ~ EcoRI

plasmid	20 ul (20 ug)
REact 3	10 ul
EcoRI	5 ul (50 units)
water	65 ul
	100 ul

10:45 ~

CDM8 cut ~ BstX I

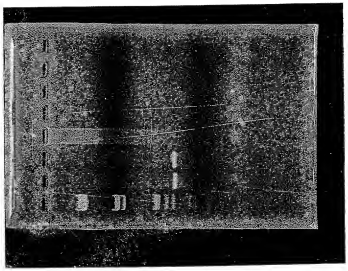
plasmid	20 ul
NEB buffer 3	10 ul
water	65 ul
BstX I	5 ul
	100 ul

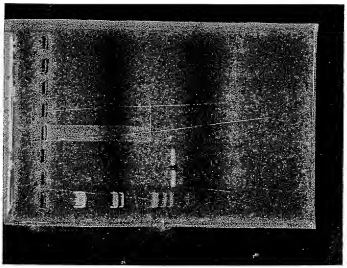
11:28 ~ at 55°C

12:20

CLP treat ÷

- 68°C 45 min in the presence of 10mM EGTA
- hot phenol 60°C extraction 5 min twice
- chloroform extraction at R.T.
- EtOH prep.





1. Negative control
2. silver-New 180 ml
3. " " 30 ml
4. " old 30 ml
5. heterozygote
6. C57BL
7. CSH
8. λ marker 5 ml (1 mg/ml)

(if concentration is 1 μg/μl) the # b × 6.6 / 2 × 10^5 = μM = pmole/μl

(3081.7 / # b)

PCR

Y0 2028 buffer 10X

Y-2010 MgCl2 50mM

Silver-old

Silver-new

C57BL

(Silver + C57BL) F1

C3H

* 30 μl reaction each × (5 reaction + 1 negative)

= 180 μl (- 6 = 174 μl)

10X buffer	18.0 μl	
MgCl2 (50mM)	5.4 μl	(1.5 mM final)
dNTP (2mM)	18.0 μl	(0.2 mM final)
primer (S1283)	1.0 μl	(0.71 pmole/μl final)
" (S1284)	1.0 μl	(0.71 pmole/μl final)
	43.4 μl	
water	129.6	
Taq polymerase	1.0 μl	(5 units)
	174.0	

divide 29 μl × 6

1. Blank 2 Silver-new 3. Silver-old 4 C57BL 5 F1 6 C3H

genomic DNA 1 μl

[redacted]

50 mg/ml final conc

Dr. Park's # 8, 18, 26 + two more : 5 Samples

Silver : 50 μl + 350 μl of TE/SDS/proteinase K buffer

→ 65°C, >1 hr. → Chloroform extraction 2 Samples
→ 2 Vol. EtOH (lagering) → spooling 3 times

Peters :

C57BL 1

C3H 1

9 Samples

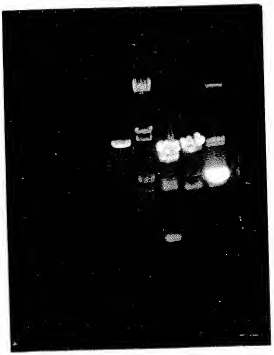
+ 1 negative control

10 Samples

· proteinase K digestion 17:05 ~ 18:05 ~ 20:25

KWON000138

① uncut Jurkat DNA
④ Jurkat DNA cut ∑ RI
③ Jurkat DNA cut ∑ RI & HIII
② λ marker 250 ng (5 ml)
⑤ CDM8 / BstXI cut. purified on 5-20% KOAc (1 ml out of 8 total)



7.5 ng / ml x 200
= 6 µg
80 ml

[redacted]

Test cut pGEM 7Zf + Junked two (in (inactivated) Sma I site)

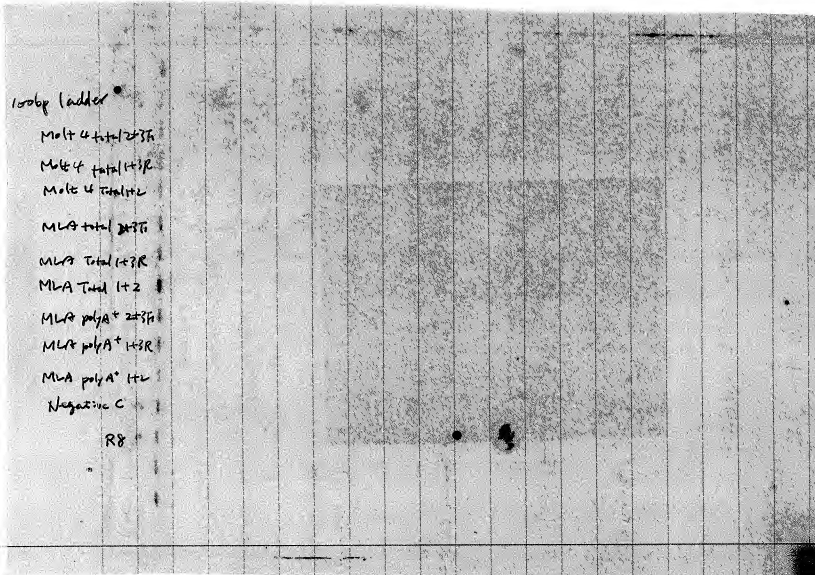
& Hind III and Eco RI

plasmid	30 µl
React 3	10 µl
water	55 µl
Eco RI	5 µl
	100 µl at 37°C (11:55 ~ 12:55)

verify cut on Agarose GE.

Cla I Restrin	100 µl
React 1	10 µl (with React 3 → becomes React 2)
water	85 µl
Hind III	5 µl
	200 µl (12:55 ~ 2:35)

- Load whole Rx mixture onto 1% Agarose
  - ↓
  - cut out band
  - ↓
  - load band onto 3.5% PAGE
  - ↓
  - purify → Nick translation


100bp ladder
Molt 4 total 2+3R
Molt 4 total 1+3R
Molt 4 Total 1+2
MLA total 2+3R
MLA Total 1+3R
MLA Total 1+2
MLA polyA+ 2+3R
MLA polyA+ 1+3R
MLA polyA+ 1+2
Negative C
R8

15

[redacted]

labelling of 4-1BB (1.2kb) by Nick-Translation

4-1BB (1.2kb)	1 ul (100ng)	1	1
NT buffer	5 ul	5	5
0.1 M DTT	2 ul	2	2
dGTP (10mM)	1 ul	1	1
dTTP (10mM)	1 ul	1 (dCTP 1)	1
$[^{32}P]$dATP	10 ul	10	10
$[^{32}P]$dCTP	10 ul	–	20
DNAase/pol	2 ul	2	2
water	18 ul	27	8
	50 ul at 16°C	1.5~2hr	4:12~ (5000)

12:42 ~ 14:20

3×10^6 cpm/ul × 100 ul × 1000ng / 100ng-ug = 3×10^8 cpm/ug

hybridization 15×20 cm NYTRAN

5M NaCl 10 ml

10% SDS 5 ml

150 μg/ml S.S. DNA (10 mg/ml × 75 μl) 75 μl

× 50 ml = 7.5 mg

Probe 3×10^6 cpm/μl [redacted] $50\text{ml} \times 10^6$ cpm/ml

$= 5 \times 10^7$ cpm

5×10^7 cpm / 3×10^6 cpm/μl ≒ 20 μl

at 65°C O/N

Wash 1. 2×SSC + 1% SDS at R.T.

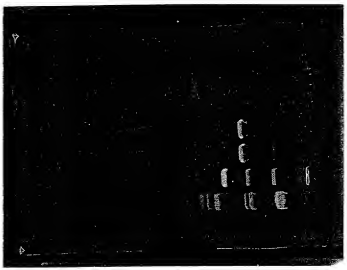
(Total 500 ml)

2. 2×SSC + 1% SDS at 42°C

for 15 min

expose film at -70°C

develop after 18 hrs



Agarose 1%

Bst XI cut (300ng)
EcoRI cut (")
uncut pcDNA1
∴ 450ng

KWON000144

[redacted]

PCDNA test cut

- dilute DNA (4 ng/μl) 1 μl in TE 19 μl (1:20 dilution)

Rx 1	diluted DNA (200ng/μl)	3 μl (600 ng)
	NEB buffer	2 μl
	water	14 μl
	BstXI	1 μl
		20 μl

50°C · 17:57 ~20:00

Rx 2	diluted DNA	3 μl (600 ng)
	React 3	2 μl
	water	14 μl
	EcoRI	1 μl
		20 μl

37°C 17:50 ~20:00

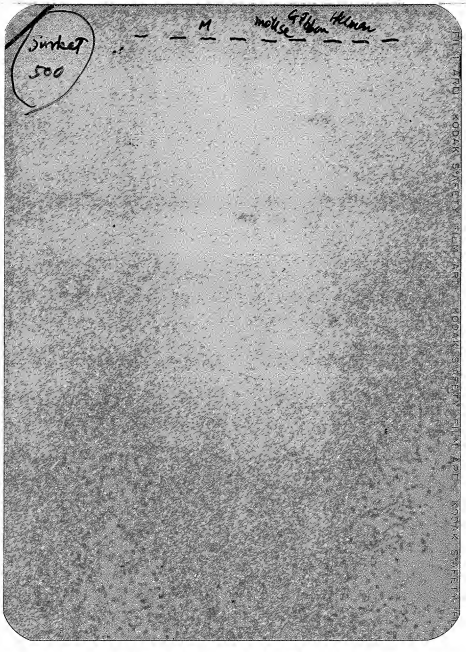
[redacted]

Membrane strip [0.2% SDS
10mM Tris pH 8.0
(50mM by default)

85°C 2h
20:40
~ 22:40

Note: Student food
helps attempting weather
to stay exposure

KWON000146


Sinkap
500
M
KODAK SAFETY FILM
KODAK SAFETY FILM

[redacted]

Nick translation of Jurkat ~~500~~ pcr fragment (PAGE purified)

DNA 1 μl (100 ng)

follows [redacted] α-[32]P labelling protocol (page 15)

at 16°C 16:25 ~ 18:25

4.7×10^6 cpm/μl × 30 μl ≅ 1.2×10^8 cpm (total)

$\frac{1. \times 10^6 \text{ cpm/μl} \times 25 \text{ μl}}{4.7 \times 10^6 \text{ cpm/μl}} = 5 \text{ μl}$

Sp. act: 1.2×10^8 cpm/μg

8 × 17.5 cm membrane : 140 cm^2 → 28 ml

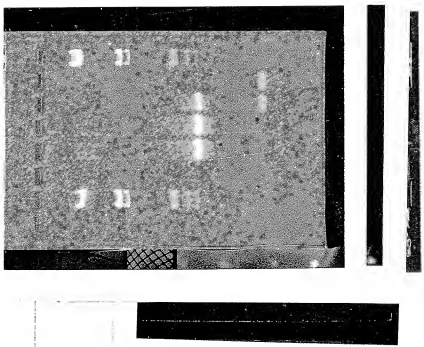
$50 \text{ ml} \times \frac{6\times}{20\times} = 15 \text{ ml}$ (of 20× SSC)

$50 \text{ ml} \times \frac{0.5\%}{10\%} = 2.5 \text{ ml}$ (of 10% SDS)

$\frac{100 \text{ μg/ml}}{10 \text{ mg/ml}} \times 50 \text{ ml} = 500 \text{ μl}$ (of 10 mg/ml SS-DNA)

Cycle profile

14	94°C	2 min				
15	94°C	1 min	55°C	1 min	72	1 min
16	94°C	"	"	"	"	2 min
17	72°C	10 min				
7	25°C					



PCR

template ① Silver
(9) ② Actin
Silver (Dr. Park's # 1, 3, 11, 26, 38) ④ ⑤ ⑦ ⑧ ⑨
③ C57BL
④ C3H

30 ml / reaction × (9 reactions + 1 negative control)
= 300 ml (– 1ul of template ×10 template = 290 ul)

Master mix

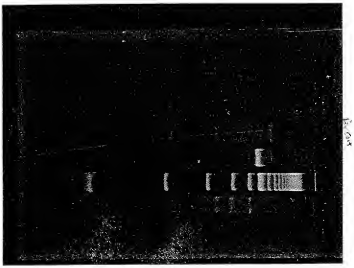
10X buffer	30.0 ul	
$MgCl_2$ (50mM)	9.0 ul	(1.5mM final)
dNTP (10mM / 5mM)	~~12.0~~ 6.0 ul	(0.2mM ")
primer (S1283)	2.0 ul	(~0.9 pmole/ul)
" (S1284)	2.0 ul	(")
Subtotal	~~49~~ 43.0 ul	
Taq	2.0 ul	(10 units)
water	~~239~~ 239.0 ul	
	290.0 ul	

- divide 29 ul into 10 tubes that contain 1ul template on the wall
- add paraffin oil (3 drops)
- Vortex → spin → cycle

MIP Brent

Brent

steel



Brent
steel
MIP

KWON000152

[redacted]

PAGE purification of Steel, Brent (pmel17), and MIP PCR

EOH ppt of 100 µl of PCR Rx. → redissolve in 20µl water
(add Glycogen or linear PA)

Steel Brent MIP ladder

[redacted]

polishing the end (aa in [redacted])

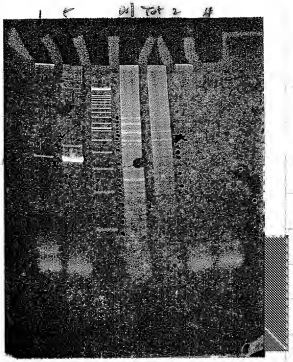
DNA → 20 µl (in D.D.W.)

10X buffer	10 µl	master mix
water	68 µl	
Kinase	1 µl	
Klenow	1 µl	
	100 µl (x 13 = 1300µl)	

80µl x 13 = 1040µl

10X buffer	130 µl
water	890
Kinase	10 µl
Klenow	10 µl
	1040 µl

(b)
8:45 ~ 9:45



1. <170, 1-2

[redacted] PCR

template

① silver ② hetero ③ C57BL ④ silver cDNA [redacted] ⑤ mouse pMZL17cDNA

100 ul/reaction × (5 reactions + 1 negative control) (half vol.)
= ~~500~~ 500 ul (= 1 ul × 5.0 ≠ ~~5.5~~)

master mix

10X buffer	~~5~~ 5 ul
$MgCl_2$ (50mM)	~~2~~ 2 ul (2mM final)
dNTP (10mM)	~~1~~ 1 ul (0.2mM ")
primer (S1283)	4 ul (~0.8 pmole/ul)
primer (S1284)	4 ul (")
subtotal	~~80~~ 76 ul
Taq.	~~3~~ 3 ul (~~0.5~~ units)
water	~~404~~ ~~448~~ 419 ~~475~~ ~~465~~

divide 99 ul each (×5) ~~+ 10 ul~~

[redacted]

Preparations for cDNA synthesis

1. pXM/RI CIP treatment

~ 20 μg pXM/RI (page 5) P/C extracted & EtOH ppt

dissolved in 90 μl of Tris (pH 8.4) according to Maniatis (pH 8.3)

aliquot 1 μl and save

add 10 μl 10X CIP buffer (10 mM ZnCl$_2$, 10 mM MgCl$_2$, 100 mM Tris (pH 8.4))

add 1 μl (1 unit/μl) of BM CIP

incubate at 37°C for 30 min.

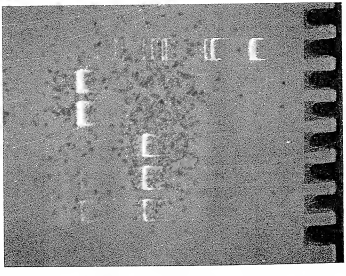
add 2 μl of 0.5M EGTA (final 10mM) and incubate at 68°C for 45 min (or 65°C for 1 hr)

add pre-treated (55°C) phenol/chloroform, vortex and incubate at 55°C for 5 min.

spin and transfer upper aq. layer to a new tube

repeat

EtOH ppt



[redacted]

PCR repeat (page 25)

template ① silver ② hetero ③ C57BL ④ silver DNA ⑤

⑤ mouse pMEL17 cDNA

Reaction volume [redacted] same as page 25

Cycle profile

1 cycle User 14 94°C 2 min

4 cycle user 15 94°C 1 min 50°C 1.5 min 72°C 2 min

11 cycle user 16 94°C 1 min 55°C 1.0 min 72°C 1 min

15 cycle user 17 94°C 1 min 55°C 1.0 min 72°C 2 min

1 cycle user 5 72°C 10 min

1 cycle user 7 25°C R.T.

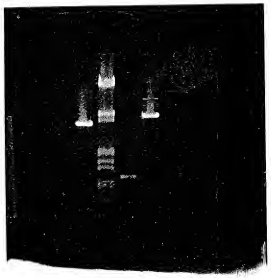
50 ul/reaction × 5 reactions

= 250 ul (- 1 ul/template × 5 template = 245 ul)

master mix	10X buffer	25 ul
	$MgCl_2$ (50mM)	7.5 ul (1.5 mM final)
	dNTP (10mM)	5 ul (0.2mM ")
	primer (S1283)	2.0 ul (1 pmole/ul)
	" (S1284)	2.0 ul (")
	subtotal	41.5 ul
	Taq	2.0 ul
	water	201.5 ul
		245.0 ul

(divide 49 ul × 5 tube
add 1 ul of template
add paraffin oil

SAMPLE	ABS(0)	ABS(1)	R^2
1.0000	0.0000	-0.0004	0.0
2.0000	0.0001	0.0001	0.0
3.0000	0.00	-0.001	0.0
4.0000	-0.00	0.00	0.0
5.0000	-0.00	0.0000	0.0
6.0000	0.0006	0.0000	0.0
7.0000	0.0004	0.0002	0.0
8.0000	0.0007	0.0000	0.0
9.0000	0.0007	0.0004	0.0



CDM 8 : Stuffer removed
G-180/R1
PXM : Some uncast remains

Test ligation of { CIP Tx PXM/R1 vector
CDM8/B;tXI

1. PXM/R1 (111 ng/μl)	1 μl	1 μl
4-1BB (15.7 ng/μl)	1.7 μl	—
5X BRL buffer	4.0 μl	4.0 μl
T4 DNA ligase	1.0 μl	1.0 μl
water	12.3	14.0 μl
	20.0 μl	20.0 μl

Vectors are not prepared well !!

↓

repurified → p39

[redacted]

Dot blot of MLA [Total RNA / poly A] PCR products

→

4-1BB	PXM	pCDM8	Ligase	Ladder	λ	poly → 98	110	120	125	150	180
210	270	240	295	370	410	470	490	550	570	600	650
poly ← Tot 700	780	270	270	510	380	410					4-1BB

3ul each of PCR product (out of 50 ul) dotted

- 4-1BB 50 ng
- PXM 100 ng
- pCDM8 300 ng
- Ladder 300 ng (0.3ul)
- λ 150 ng

1. after application float on D.D.W
2. denature for 5'
3. neutralization for 5'
4. rinse in 2XSSC
5. partially dried → Stratalinker

SAMPLE	A320	A[illegible]	A[illegible]	[illegible]	[illegible]	[illegible] DNA:D.W		NUCLEIC ACID	
1.0000	0.0000	[illegible]	0.0000	******	******	[illegible]		[illegible]	
2.0000	0.0188	0.0399	0.0562	0.4464	2.2401	pMELI7/PVUII [illegible]	6:54	2.2[illegible]	22.197 ng/µl
3.0000	0.0000	0.0000	0.0000	******	******	[illegible]		0.000[illegible]	
4.0000	0.0050	0.1120	0.2143	0.5082	1.9753	pCDM8 [illegible]	6:66	[illegible]	97.536 ng/µl
5.0000	0.0071	[illegible]	[illegible]	0.0452	[illegible]	[illegible]		[illegible]	
6.0000	[illegible]	0.0028	[illegible]	[illegible]	[illegible]	[illegible]		0.062[illegible]	
7.0000	0.0013	0.0000	[illegible]	1.0000	[illegible]	[illegible]		0.034[illegible]	
[illegible]	0.0052	0.0432	[illegible]	0.5005	1.9979	pcDNA1 [illegible]	6:54	3.414[illegible]	34.146 "
[illegible]	0.0000	[illegible]	0.0000	******	******	[illegible]		0.0000	
[illegible]	0.0161	[illegible]	[illegible]	0.5128	1.950[illegible]	pXM [illegible]	6:54	[illegible]	27.858 "

KWON000162

[redacted]

ligation of BstXI cut pCDM8 & pCDNA1
with adapted pVIII fragment of pMZL17 (: or pXM frag)

1. Adaptor ligation

pVIII fragment (22 ng/μl)	2 μl
BstXI adapter (0.5 μg/μl)	1 μl
5X BRL lig. buffer	4 μl
water	12 μl
T4 ligase	1 μl
	20 μl at 16° / hour

11 : 00 a.m

- at 65°C 10 min
- add NaI (gene clean kit) 150 μl
- add 2 μl of glassmilk (01:17)
- follow gene clean procedure
- elute twice → total 20 μl

CDNA

Transfo |

Invitro 0.3ml

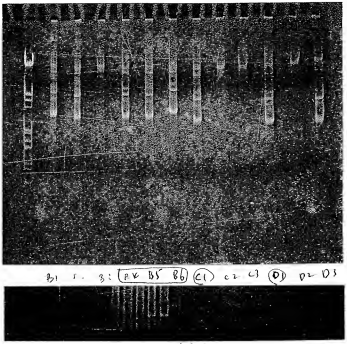
add (0.3ml)

divi μl

on

add tube

cleaned)



on

heat shock at 42°c water bath for 65 seconds

on ice for > 2 min

add ~~250~~ 300 μl of SOC medium (provided by Invitrogen)

37°c on the wheel for 1 hr.

plate whole thing on Amp-LB plate

(g. h; before plating add 3ml LB and plate 100 μl each)

100ng vector

25ng

40

104000

1 x 10^5/μg

a: nothing ~~pCDNA 8~~ 261

b: ~2600 pcDNA1 f: 205

c: 335 g: ~560 × 325 = 18,200/ng

d: 279 h: nothing

miniprep super [g, h

a, e, g cDNA 8 both in pCDNA

10^7/ug

KWON000164

Ligation of adapter-pmel17/pUC12 to [CDM8 / pCDNA1]

1. gene-cleaned adapter-pmel17/pUC12 ~ 10 µl (~20 ng)

ⓐ CDM8 (87 ng/µl)	ⓑ pCDNA1 (34 ng/µl)	1 µl / 3 µl
5X lig. buffer (BRL)		4 µl / 4 µl
water		Vector alone 4 µl / # self-ligate (16) 2 µl / (12) 1 µl
ligase (T4 DNA ligase, BRL)	ⓒ	1 µl / 1 µl
		20 µl / 20 µl

at 16°C

※ control : pmel17/pUC12 in place of adapter-pmel17/pUC12

pmel17/pUC12 (22 ng/µl)		1 µl / 1 µl
ⓒ CDM8 (97 ng/µl)	ⓓ pCDNA1	1 µl / 3 µl
5X lig. buffer		4 µl / 4 µl
water		13 µl / 11 µl
ligase		1 µl / 1 µl
		20 µl / at 16°C

2. Transform [CDM8 / pCDNA1] × [vector alone; vector + frag.; vector + adapter + frag.; uncut vector (1 ng)]

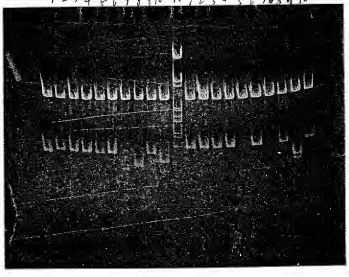
(8 colonies)

ⓒ CDM8 ⓓ pCDNA1

Ligation of pXM/RI · CIP

1. pXM/RI (78 ng/μl) CIP	2 μl	2 μl	1 μl
4-1BB (16 ng/μl)	2.5 μl	—	—
5X BRL lig. buffer	4 μl	4	4
water	10.5	13 μl	14
T4 ligase (BRL)	1 μl	1	1
	20.0 μl	20 μl	20 μl

* pXM/RI CIP - not Tx 1 μl



MHz 500 : 1, 2, 3, 4, 6, 8, 10

: 9

: 5, 7 (no insert)

MHz 400 : 1

: 2, 3, 4, 5, 6, 9

: 7

: 8

: 10

[redacted]

digestion of MIP400 & MIP300 clones (10 each X 2)

- mastermix I for 20 ml x 20 = 400 (- 5 ml of miniprep = 395)

React 3	40 ml
water	240 ml
EcoR1	20 ml
	300 ml

- divide into 20 used & washed tubes
- add 5 ml of minipreps
- mix and at 37°C for 2hr
- take 10 ml separate

Into remaining 10 ml add 10 ml of mastermix 2

mastermix 2

React 1	20 ml
water	170
Hind III	10 ml
	200 ml

mix and incubate for 1 hr at 37°C

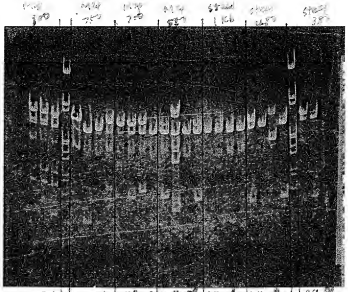
take 10 ml and run gel

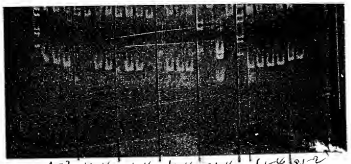
* Transform XL-1 blue ⓒ ligation mixture of
  polished PCR products of page 27 & 47

page 27 (steel 1kb, 480, 180
  MLP 500, 750, 700, 550

page 47 (⟹)

* pick 4 colonies ~~xxxx~~ from each plate
  prepare plasmid
  digest with





[redacted]

ligation of pcr products
- Steel 1kb, 480, 380
- MLP 650, 700, 750, 900

(7 fragment)

ligation

~~77~~ 20ul = 140 ul (- ~~[redacted]~~ 10ul × 7 = 70ul)

5x buffer	28 ul
vector	1 ul (pEGFN3/smu2 CTP tx)
water	36 ul
T4 ligase	5ul
	70 ul

divide into 7 tubes (10ul each)
add pcr frag. (1.2ul each)
at 20°C 65°C inv

[redacted]

ligation of pcr products from [redacted] and [redacted]

[redacted] (page 29) Silver genomic 1-2 (350bp)
mouse pMEL17 cDNA 350bp 5-1, 450bp 5-2

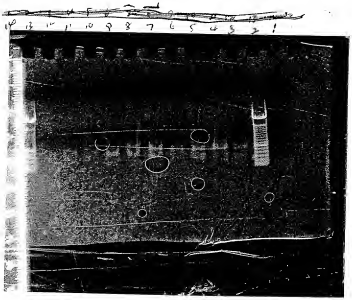
[redacted] (page 33) silver cDNA 4 (350bp)
silver genomic 1 (1.2kb and 350bp)

6 × 20 ul = 120 ul (- 10 ul × 6 = 60 ul)

5X buffer	24 ul
vector	1 ul
water	32 ul
T4 ligase	3 ul
	60 ul

divide into 6 tubes 10 ul each
add ⊗ repaired frag.
at 20°C

a 1-2 half (10ul)
b 5-1 2 ul
c 5-2 half (10ul)
d 4 2ul
e 1 (1.2kb) half
f 1 (350) half



prehybridization

6X SSC

5X Denhardt

1% SDS

150 ug/ml SSDNA

at 7:20 at 65°C

8:20

hybridization

6X SSC

5X Denhardt

1% SDS

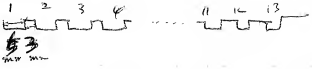
100 ug/ml SS DNA

4-1BB probe 5×10^6 cpm/ml

at 37°C

KWON000171

[redacted]



total
101 mm

1. ~~ladder~~ 4-1BB
2. ~~4-1BB~~ ladder
3. 98 220 530
4. 110 240 570 poly A+ mix 7ul ~~of each~~ frag
5. 120 295 600
6. 135 320 650
7. 150 410 700
8. 180 470 780
9. 210 490
10. 220 380 total RNA
11. 270 410
12. 350
13. ~~4-1BB~~ ladder
14. 4-1BB

SAMPLE	A320	A280	A260	280/260	260/280	PROTEIN	NUCLEIC ACID	
1.0000	-0.001	0.0000	0.0010	0.5095	1.9615	0.0692	0.0309	
2.0000	0.0045	0.0424	0.0801	0.4967	2.0043	1.2321	3.3724	pRC/CMV (BstXI) = water
3.0000	-0.001	-0.001	0.0000	-0.080	-12.50	-0.981	0.0650	2.5 : 57.5 ⇒ 84 ng/μl
4.0000	0.0293	0.0526	0.0678	0.5894	1.6966	0.0585	1.8039	
5.0000	-0.001	0.0000	0.0000	1.0000	1.0000	0.9536	0.0328	
6.0000	0.0119	0.0201	0.0300	0.4523	2.2108	-0.997	0.8410	
7.0000	0.0112	0.0205	0.0295	0.5698	1.7614	0.6213	0.9143	
8.0000	-0.002	-0.002	-0.002	-2.000	-0.500	-0.443	0.0216	
9.0000	0.0174	0.0338	0.0498	0.5222	1.9146	1.6751	1.3883	pMEL 17-1/pVUE BstXI; water
10.000	0.0181	0.0340	0.0495	0.5077	1.9692	0.9589	1.3774	5 : 55 ⇒ 16.8 ng/μl

[redacted]

cut pRC/CMV with BstXI

plasmid	15 ul (15 ug)
NEB #3	10 ul
water	70 ul
BstXI	5 ul
	100 ul at 50°C [illegible] 90

~~add BstXI~~

ligation

	①	②	③	④	⑤	⑥
pCDM8	1 ul	1 ul	—	—	—	—
~~pRC/CMV~~ ~~PCNA1~~	—	—	1	1	—	—
~~pCDNA1~~ ~~RSV/CMV~~	—	—	—	—	2.5	2.5
5X lig. buffer	4	4	4	4	4	4
prep 17 pVUII/BstXI	1	—	1	—	1	—
water	13 (13.5)	14 (14.5)	13 (13.5)	14 (14.5)	11.5	12.5 (11.5+1)
ligase	1	1	1	1	1	1
	20	20	20	20	20	20

④ : ⑤ (7)

Master mix 20 × 6 = 120 {−(1+4.5) × 6 = ~~99~~}

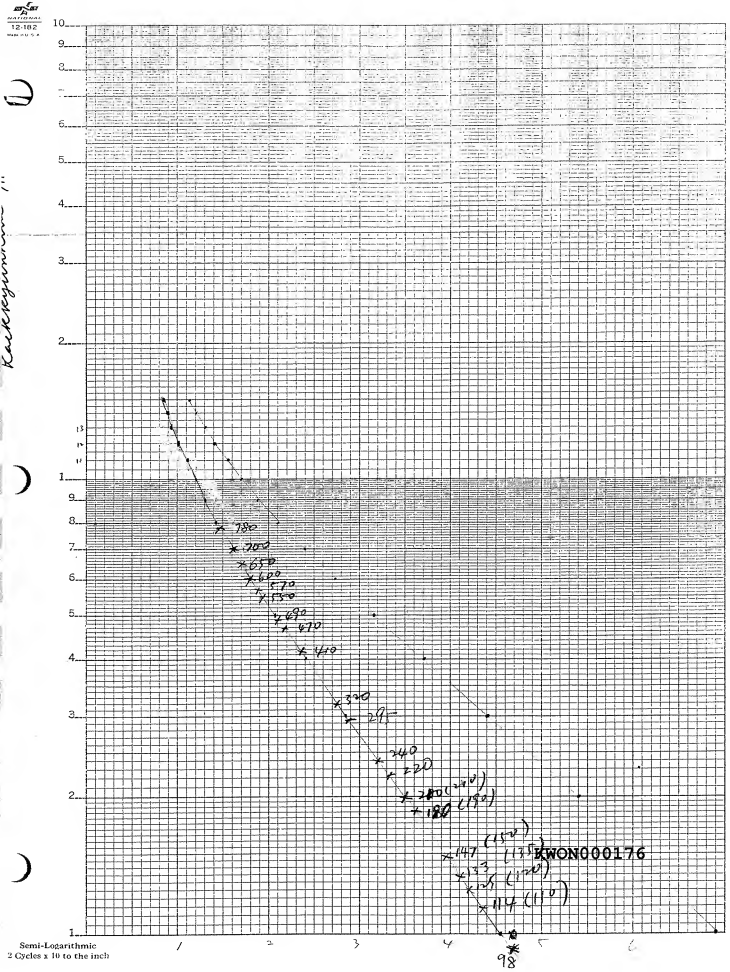
5x buffer	24
water	~~69~~
ligase	6
	~~99~~ ul (16.5)

KWON000174

To do

1) stuffer 1 kb.
   Dr. Kim has ligate
   plate XL-1 blue

2) all the fragment of MIP-PCR
   stuffer have been repaired
   & cloned


780
700
650
600
570
550
690
670
410
320
290
240
220
200 (190)
180
147 (150)
133 (120)
114 (110)
98
KWON000176
Semi-Logarithmic
2 Cycles x 10 to the inch

KWON000177